Hiromasa Ohira *Editor*

Autoimmune Liver Diseases

Perspectives from Japan

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Springer

Editor
Hiromasa Ohira
Department of Gastroenterology and Rheumatology
Fukushima Medical University School of Medicine
1 Hikarigaoka, Fukushima 960-1295, Japan

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Preface

The liver is a unique organ that can induce immune tolerance but can also be affected by autoimmune disorders. For this book, we invited leading Japanese scientists in the field to outline recent advances in autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), the two major autoimmune liver diseases caused by immune disorders. It is our great pleasure to have the opportunity to introduce to the world's readers the current clinical approaches in treating AIH and PBC in Japan as well as various clinical challenges and research results.

Recent epidemiological studies on AIH have raised questions about the pathogenesis, medical therapy, and treatment outcomes of the condition. In response to this trend, new approaches for analyzing and interpreting its etiology and pathology, including new animal models, have been introduced. Although most Japanese cases of AIH are responsive to steroid therapy, some cases undergo a relapse of inflammation during steroid reduction. Furthermore, some cases even present acute hepatitis-like manifestations in the form of severe hepatitis or acute liver failure (fulminant hepatitis, late-onset liver failure), which can be treatment-refractory. Recent studies have revealed that the pathogenesis of acute hepatitis-like manifestations includes two phases: the acute exacerbation phase and the acute hepatitis phase. Patients in the acute hepatitis phase may have low serum IgG levels and negative or low autoantibody titers, making diagnosis difficult. Attention has also been paid to the treatment of mild and elderly cases, including cases in which steroid treatment can be discontinued, cases in which there is an overlap with nonalcoholic steatohepatitis (NASH), and cases of IgG4-related AIH.

Concerning PBC, a study group of the Ministry of Health and Welfare (currently The Ministry of Health, Labour and Welfare) has been conducting an epidemiological study since 1980 and has created one of the world's largest databases including more than 8,500 cases of PBC thus far. Moreover, taking advantage of the homogeneous racial population of Japan, prognosis prediction by analyzing SNPs and autoantibodies, such as anti-gp210 and anti-centromere antibodies, has become increasingly feasible. The recent introduction of genome-wide association study (GWAS) has also contributed to the generation of research findings that are more relevant to the pathogenesis of the disease. There has also been an

accumulation of data on the effectiveness of bezafibrate. New pathological staging/activity grading criteria and clinical practice guidelines have also been established. The world's first case of adult living-donor liver transplantation was performed in Japan in a patient with PBC. Unlike in Western countries, most cases of PBC undergo living-donor liver transplantation as the first-line treatment in Japan, with favorable outcomes.

I hope that this book will be helpful in facilitating clinical and research activities on autoimmune liver diseases. Finally, I would like to thank all of the authors for their contributions as well as Springer Japan for their efforts in publishing this book.

Fukushima, Japan Hiromasa Ohira

Contents

Part I Autoimmune Hepatitis

1 Pathogenesis of Autoimmune Hepatitis 3
Hiroki Takahashi

2 Models of Autoimmune Hepatitis 21
Norihiko Watanabe and Aki Ikeda

3 Epidemiology and Natural History in Japan 37
Kaname Yoshizawa, Akihiro Matsumoto, and Takeji Umemura

4 Histological Findings of Autoimmune Hepatitis 45
Kenichi Harada

5 Diagnosis of Autoimmune Hepatitis 67
Mikio Zeniya

6 Acute Presentation of Autoimmune Hepatitis 83
Masanori Abe and Morikazu Onji

7 Treatment of Autoimmune Hepatitis 95
Yasuhiro Miyake

8 Management Strategies for Autoimmune Hepatitis Treatment Nonresponders 107
Teruko Arinaga-Hino, Tatsuya Ide, and Michio Sata

9 Pediatric Autoimmune Hepatitis 121
Tomoo Fujisawa

10 Nonalcoholic Steatohepatitis-Autoimmune Hepatitis Overlap 127
Atsushi Takahashi, Kazumichi Abe, and Hiromasa Ohira

11 IgG4-Related Autoimmune Hepatitis 137
Takeji Umemura

Part II Primary Biliary Cirrhosis

12 The Onset Mechanism of Primary Biliary Cirrhosis 147
Shinji Shimoda

13 Genetic Factors in the Pathogenesis of Primary Biliary Cirrhosis 157
Minoru Nakamura

14 Animal Models for Primary Biliary Cirrhosis 171
Yuki Moritoki and Yoshiyuki Ueno

15 Epidemiology and Natural History in Japan 201
Junko Hirohara, Toshiaki Nakano, Toshihito Seki, Kazuichi Okazaki, Kenichi Harada, Hiromi Ishibashi, Yasuni Nakanuma, and Hirohito Tsubouchi

16 New Histological Staging and Grading System for Primary Biliary Cirrhosis 215
Yuko Kakuda, Kenichi Harada, and Yasuni Nakanuma

17 Autoantibodies in Primary Biliary Cirrhosis 233
Atsumasa Komori

18 Diagnosis and UDCA Treatment of Primary Biliary Cirrhosis 249
Atsushi Tanaka

19 Bezafibrate Treatment of Primary Biliary Cirrhosis 261
Shinji Iwasaki

20 Management of the Patients with Feature of Autoimmune Hepatitis 277
Kazumichi Abe, Atsushi Takahashi, and Hiromasa Ohira

21 Liver Transplantation for Primary Biliary Cirrhosis 287
Takuya Genda and Takafumi Ichida

Index 301

Part I
Autoimmune Hepatitis

Chapter 1
Pathogenesis of Autoimmune Hepatitis

Hiroki Takahashi

Abstract Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease and its pathogenesis is characterized by an autoimmune reaction to hepatocytes. Initiation of the disease can be related to both environmental and genetic factors, and AIH is likely to develop in people with predisposed genetic backgrounds who have been exposed to conducive environmental factors, such as viral infections or drugs. In autoimmune diseases, autoantigens stimulate autoreactive cytotoxic T cells and induce a pathological autoimmune reaction; however, AIH-specific autoantigens and mechanisms of onset remain unknown. Under normal physiological conditions, autoimmune reactions are negatively regulated by immunological tolerance and the loss of tolerance is an important component in the pathogenesis of AIH. The loss of tolerance may be caused by abnormalities of regulatory cells such as Foxp3-positive $CD4^+$ T cells, which participate in the maintenance of tolerance. Additionally, the interaction among several kinds of immune cells (dendritic cell, T cell, B cell, natural killer cell, and natural killer T cell), hepatocytes, and non-parenchymal cells (Kupffer cell, hepatic stellate cell, and sinusoidal endothelial cell) may also participate in the pathogenesis. Identification of the autoantigens as well as the cellular interactions involved is critical for a complete understanding of the pathogenesis of AIH.

Keywords Autoantigen • Autoimmune hepatitis • Effector cells • Pathogenesis • Regulatory T cells

H. Takahashi (✉)
Gastroenterology, JIKEI Graduate School of Medicine, 3-25-8 Nishishinbashi, Minato-ku, Tokyo 105-0003, Japan
e-mail: hiroki@kk.iij4u.or.jp

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1.1 Introduction

1.1.1 Overview of the Pathogenesis of Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease that is characterized by an autoimmune reaction to hepatocytes. Although its pathogenesis has been extensively studied for a long time, the detailed mechanisms are unknown and many questions remain (Fig. 1.1).

1. What are the *genetic factors* that relate to the susceptibility and disease progression of AIH?
2. What are the *environmental factors* that relate to the initiation of AIH?
3. How does the *innate immune response* relate to the pathogenesis of AIH?
4. How do antigen-presenting cells, such as dendritic cells, activate autoantigen-specific T cells?
5. What is the disease-specific *autoantigen*?
6. How are the autoantigens released from hepatocytes and captured by antigen-presenting cells?
7. How is the *immune tolerance* of autoreactive T cells broken?

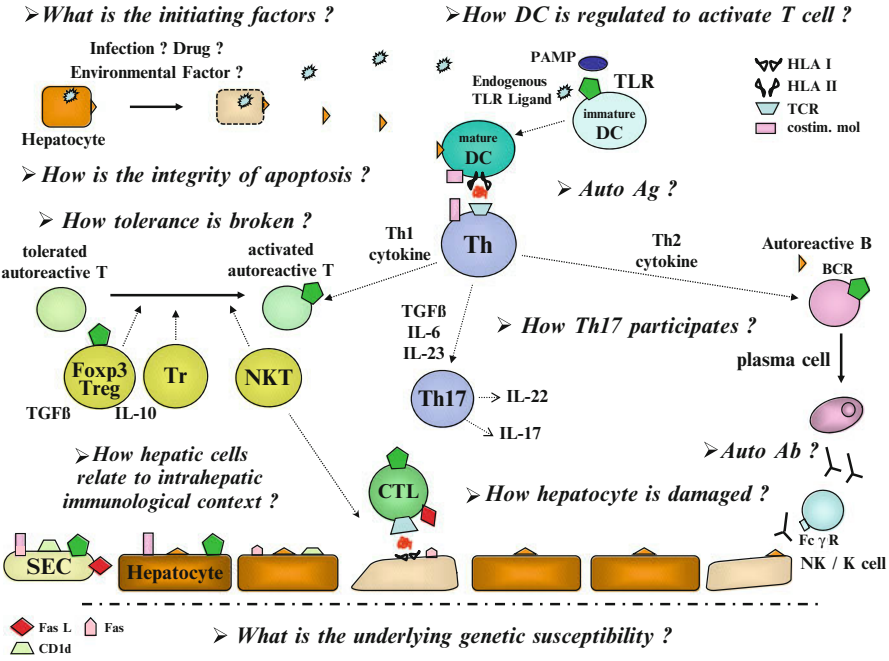

Fig. 1.1 Unsolved questions in the pathogenesis of AIH

8. Do abnormalities of frequency and function of *regulatory T cells* exist? And how do these cells relate to the loss of the immune tolerance to autoreactive T cells?
9. How do effector cells damage hepatocytes?
10. How do effector cells migrate into the liver?
11. How are autohepatocytes damaged by effector cells?
12. How do non-parenchymal cells participate in the pathogenesis of AIH?
13. Do *autoantibodies* participate in hepatocyte damage?

1.1.2 The Events and Factors That Relate to the Pathogenesis of AIH

The pathogenesis of AIH is considered to follow for sequential events.

1.1.2.1 Initiation of Disease

The first event is the initiation of disease and both environmental and genetic factors may participate. AIH may develop when people with a disease-specific genetic background are exposed to conducive environmental factors such as viral infections or drug.

1.1.2.2 Appearance of an Autoantigen and the Development of Autoimmune Reaction

The second event is the autoimmune response to an autoantigen on hepatocytes, including the production of autoantibody. The appearance of autoantigen is considered the most important event in the pathogenesis of AIH; unfortunately, the AIH-specific autoantigen has not been identified and the underlying mechanism of its appearance has not been analyzed. Currently, this is the biggest challenge in the research of the pathogenesis of AIH.

Under normal physiological conditions, autoimmune reactions are negatively regulated by immunological tolerance. The loss of immunological tolerance occurs in AIH by unknown mechanisms. Recent progress in basic immunology research has revealed that immunological tolerance is maintained by regulatory T cells such as Foxp3-positive CD4 T cells (Tregs). It is possible that abnormalities in the number and/or function of regulatory cells may result in the loss of immunological tolerance in AIH.

1.1.2.3 Damage to Hepatocytes

The third sequential event is the actual damage to hepatocytes mediated by effector cells. Autoantigen-specific *cytotoxic T cells* (CTLs) are considered primary effector cells that damage hepatocytes. However, recent studies of another organ-specific autoimmune disease such as rheumatic arthritis indicate that other effector cells, such as Th17 cells and NKT cells, can also participate in the pathogenesis of disease.

1.1.2.4 Maintenance of Chronic Inflammation

Finally, the last step is the maintenance of chronic intrahepatic inflammation. Several factors are involved in chronic inflammation including endogenous danger molecules, which are derived from dying cells and considered to stimulate innate cells. Nonetheless, the precise mechanisms controlling the chronic autoreactive response in AIH remain unknown.

1.2 Environmental and Genetic Factors Related to the Initiation of AIH

AIH is considered initiated when people with predisposed genetic backgrounds are exposed to conducive environmental factors, such as viral infections or drugs.

1.2.1 Environmental Factors

The most well-described environmental factors related to the initiation of AIH are viral infections and drugs (Table 1.1).

1.2.1.1 Viruses

The viruses reported to initiate the development of AIH includes measles virus, hepatitis A virus, Epstein–Barr (EB) virus, herpes virus, and cytomegalovirus [1].

1.2.1.2 Drugs

Several drugs including oxyphenisatin, methyldopa, nitrofurantoin, diclofenac, interferon, pemoline, and minocycline have all been reported to initiate the

Table 1.1 Environmental factors that initiate AIH

Virus
Hepatitis A virus
EB virus
Herpes virus
Hepatitis B virus
HIV
Drugs
Statin
Atorvastatin, simvastatin
Others
Oxyphenisatin, methyldopa, nitrofurantoin, diclofenac
Interferon, pemoline, minocycline

development of AIH. Recently, several cases of patients who developed AIH after taking statins, such as atorvastatin and simvastatin, have also been reported [2].

1.2.1.3 How Do Environmental Factors Initiate AIH?

The mechanisms by which environmental factors initiate AIH remain largely unknown. There is speculation that interactions between drug metabolites or element of virus and the hepatocyte membrane create a neo-antigen, which becomes the autoantigen driving AIH. Furthermore, these metabolites or particles could stimulate immune cells via *toll-like receptors* (TLR) or intranuclear receptors signaling and induce immune activation. Such antigen-nonspecific immune activation may contribute to the induction of the antigen-specific autoimmune response.

1.2.2 Genetic Background

Genetic factors are considered to contribute to the susceptibility of AIH and disease progression as well as response to therapy, and human leukocyte antigen (HLA) has long been studied in this context. Recently, single nucleotide polymorphisms (*SNPs*) have also been investigated in known potential disease-specific susceptibility genes as well as genome-wide association study (GWAS), which analyzes SNPs throughout the genome to identify disease-specific susceptibility genes.

1.2.2.1 HLA

The most well-described disease-specific susceptibility gene is HLA. *HLA-DR4* (DRB1*0405) is a disease-specific susceptibility gene that is observed in approximately 60 % of Japanese patients with AIH [3]. In addition to disease susceptibility, it is clear that HLA-DR alleles relate to prognosis and response to therapy.

Table 1.2 SNPs as susceptibility gene of AIH

CTLA-4 (exon 1 + 49)
Yes: UK, China
No: Brazil, Germany, Japan
TNF-α (promoter −308)
Yes: UK (DRB1*0301)
No: Brazil, China
VDR (exon 2)
Yes: Germany, China

DRB1*04 relates to prognosis, and, compared with patients with other HLA-DR alleles, DRB1*13 which is frequently observed in patients who are negative for DRB1*04 positively correlates with response to therapy in those patients carrying the DRB1*13 allele and who do not relapse during maintenance therapy.

Interestingly, ethnic differences between Japanese and Caucasian patients with AIH are observed in the HLA genotype. In Caucasian patients, HLA-DR3 (DRB1*0301) is most frequently observed, followed by DR4 (DRB1*0401). HLA-DR3 is not observed in Japanese patients. There is also a difference in susceptible age and disease activity between patients with DR3 and patients with DR4 among Caucasian patients. Patients with DR3 are young and have severe disease activity, whereas those with DR4 are middle-aged or elderly and have mild disease activity. Because Japanese patients with DR4 are middle-aged or elderly and have mild disease activity, the effect of HLA-DR4 as a susceptibility gene may reach beyond ethnic differences.

1.2.2.2 SNPs

Genetic polymorphisms have also been studied as candidates of disease-specific susceptibility in AIH. SNPs within particular transcripts such as *cytotoxic T lymphocyte-associated antigen-4* (*CTLA-4*) on chromosome 2 [4], tumor necrosis factor-α (TNF-α) on chromosome 7 [5], and *1,25-dihydroxyvitamin D receptor* on chromosome 14 [6] have been reported to confer disease-specific susceptibility in patients with AIH. SNP within CTLA-4 has also been reported to confer susceptibility to other autoimmune liver diseases, including primary biliary cirrhosis (PBC). However, the results of SNP studies differ from countries and are still controversial (Table 1.2).

1.2.2.3 Genome-Wide Association Study (GWAS)

GWAS, which analyzes SNPs throughout the genome, is performed to identify disease-specific susceptibility genes in several autoimmune diseases. In PBC, GWAS of Japanese patients identified SNPs in several molecules, in the immunological pathway as disease-specific susceptibility genes [7]. GWAS of Japanese

Table 1.3 Epigenetic changes

Methylation of DNA
Modification of histone protein
Substantively change of messenger RNA by microRNA

patients with AIH is currently in progress and is expected to yield new and valuable information on the mechanism of this disease.

Thus the influence of SNPs on the function of a molecule is an important point to remember. When disease-specific SNPs are identified, it will be critical to analyze how the genetic change influences the expression or function of the protein to completely understand how SNPs participate in the pathogenesis of diseases.

1.2.3 Epigenetic Changes

While the importance of environmental factors that initiate autoimmune diseases has been recognized, the underlying mechanisms remained unknown for a long time.

Recently, epigenetic changes such as DNA methylation, histone modification, and substantive changes in messenger RNA by microRNAs, which alter gene expression and/or function without modifying the base sequence, have been linked to environmental factors (Table 1.3). Presumably, an autoimmune response may be the result of epigenetic changes, which are induced by environmental factors, in genes related to immune function in immune cells or non-parenchymal cells. Analysis of epigenetic changes in immune cells and non-parenchymal cells will give us new knowledge regarding the interaction between environmental and genetic factors that participate in the pathogenesis of AIH.

1.3 The Role of Innate Immune Response in the Pathogenesis of AIH

Recent advances in basic immunology have revealed the mechanisms of innate immune response that recognize pathogen-associated molecule patterns (PAMP) using pattern-recognizing receptors (PRRs) such as TLRs on immune cells.

The innate immune response mainly participates in infectious immunity such as antibacterial and antiviral responses. Innate immune responses also participate in inflammatory responses in the absence of infection. For example, damage-associated molecular patterns (DAMPs) such as nucleic acid or uric acid, which are released from necrotic cells, also stimulate the innate response by binding to TLRs expressed on immune cells, thereby inducing inflammatory responses through the activation of the inflammasome pathway or cytokine production.

Table 1.4 How innate immune response participates to the pathogenesis of AIH

Does it participate in the initiation phase?
Does it participate in the chronic inflammatory phase?

Although detailed mechanisms remain unknown, such phenomenon may participate in the initiation phase and/or chronic inflammatory phase of AIH (Table 1.4).

1.4 The Role of Acquired Immunity in the Pathogenesis of AIH

Generally, the primary pathogenesis of autoimmune diseases is the autoantigen-specific acquired immune response. There are several essential concepts that need to be understood in the autoantigen-specific acquired immune response in AIH.

1.4.1 What Is an Autoantigen?

In type II AIH, which is rarely found in Japanese patients, CYP2D6 has been identified as the disease-specific autoantigen [8]. Furthermore, the epitopes that are recognized by autoreactive T cells have also been identified [8]. In contrast, the disease-specific autoantigen of type I AIH, which is frequently found in Japanese patients, has not yet been identified. In fact, the disease-specific autoantibody of type I AIH is an antinuclear antibody, which likely recognizes several antigens.

1.4.2 How Is the Autoantigen Revealed?

The mechanisms of the appearance of autoantigen also remain unknown (Fig. 1.2).

1.4.2.1 *Neo-antigen* Induced by Initiation Factors

It is hypothesized that the interaction between drug metabolites or viral particles and the hepatocyte membrane leads to the formation of a neo-antigen, which then becomes the autoantigen.

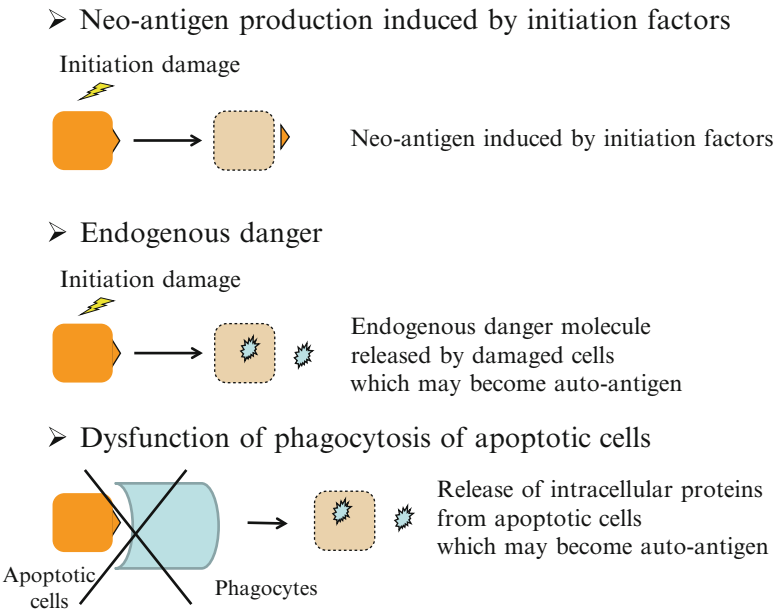

Fig. 1.2 How does an autoantigen appear?

1.4.2.2 Endogenous Danger Molecules

Intracellular proteins released by damaged cells may work as "endogenous danger molecules" and become autoantigen.

1.4.2.3 Dysfunction of Phagocytosis of Apoptotic Cells by Phagocytes

Another possibility is that dysfunction of phagocytosis of apoptotic cells by macrophages may lead to release of intracellular proteins that may become autoantigens.

1.4.3 How Autoreactive T Cells Activated?

1.4.3.1 Tolerogenic Intrahepatic Dendritic Cells in Steady State

The liver is a unique organ that has the ability to induce immune tolerance. Intrahepatic *dendritic cells* (*DCs*) exist in an immature condition and induce immune tolerance as tolerogenic DCs in physiological condition [9]. Efficacy of

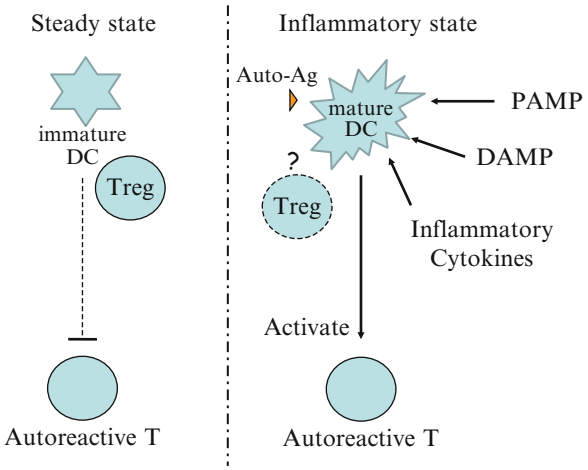

Fig. 1.3 How are intrahepatic DCs activated?

tolerance induction by intrahepatic DCs compared with DCs that exist in other organs has also been reported. There is a possibility that regulatory T cells such as Foxp3-positive regulatory T cells (Tregs) cooperate to maintain intrahepatic immune tolerance (Fig. 1.3).

1.4.3.2 Activated DCs Activate Autoreactive T Cells in Inflammatory State

Once intrahepatic inflammation occurs, intrahepatic DCs are activated and stimulate T cells by presenting antigen (Fig. 1.3). Although the detailed mechanisms controlling the activation of intrahepatic DCs remain unknown, there is the possibility that PAMPs or DAMPs, which appear in intrahepatic inflammatory condition, stimulate a conversion from tolerogenic DCs to powerful antigen-presenting cells. Inflammatory cytokines such as IFN-γ or IL-12, which are produced in inflammatory conditions, may also activate DCs. Activated DCs may phagocytose the autoantigen released by hepatocytes and present it to autoreactive T cells. The reports that demonstrated higher expression of activated *co-stimulatory molecule* B7-H1 on intrahepatic DCs in patients with AIH compared with healthy control support the activated state of intrahepatic DCs [10]. Dysfunction of Tregs, which will be discussed later, may also contribute to the activation of DCs.

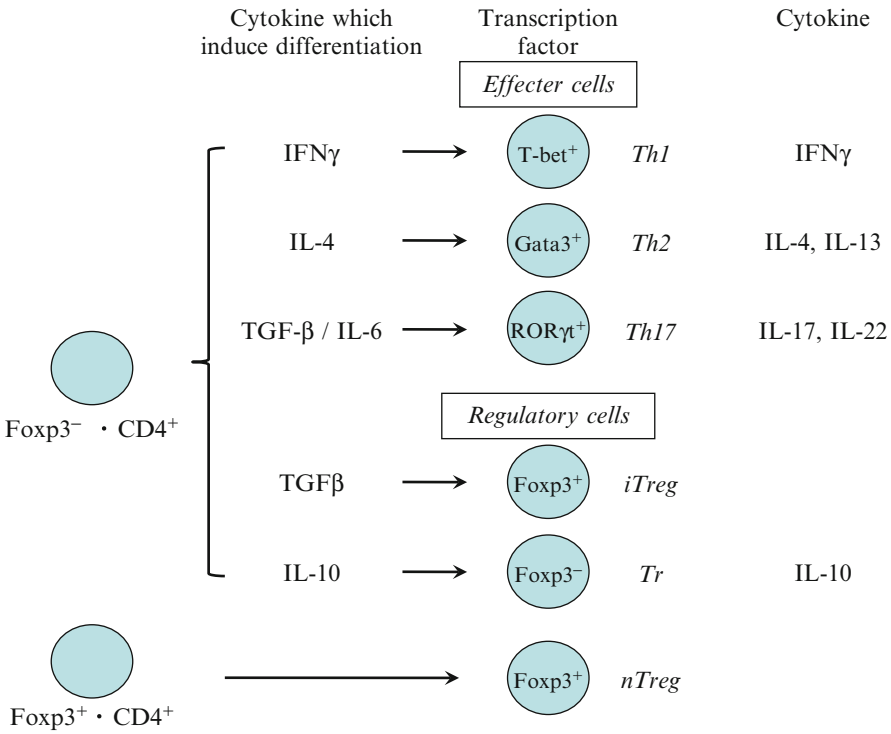

Fig. 1.4 The differentiation of CD4-positive T-cell subsets

1.4.4 What Are the Effector Cells That Damage Hepatocytes?

1.4.4.1 Cytotoxic T Cells

Because the intrahepatic infiltration of CD8-positive T cells is frequently observed in patients with AIH, CD8-positive cytotoxic T cells (CTLs) are thought to participate in damage of hepatocytes. CTLs are activated by inflammatory cytokines such as IFN-γ or IL-12, which are produced in inflammatory conditions.

1.4.4.2 Th17 Cells

Several effector and regulatory cells have been identified to be subsets of differentiated CD4-positive T cells. The cytokines that induce each subset and the transcription factors that regulate their differentiation have also been identified (Fig. 1.4). Recent reports of other organ-specific autoimmune diseases have revealed that Th17 cells, which are a newly identified CD4-positive T-cell subset

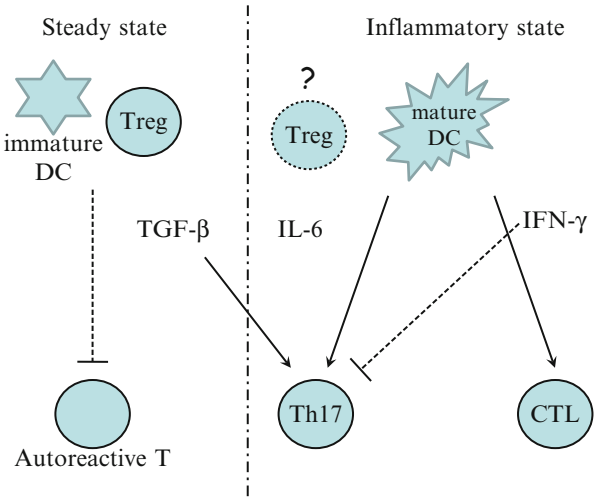

Fig. 1.5 Mechanisms of the induction of CTLs or Th17 cells

that produce IL-17, also work as effector cells. In AIH, increases in serum levels of IL-17 as well as the frequency of intrahepatic Th17 cells have been reported [11]. However, it is not clear whether Th17 cells work as effector cells in the pathogenesis of AIH.

Whether CTLs and Th17 cells work with each other as effector cells in AIH also remains unclear. Interestingly, CTLs are induced by *IFN-γ*, and Th17 cells, which are induced by *TGF-β* and *IL-6*, are inhibited by IFN-γ (Fig. 1.5). These findings indicate that the intrahepatic cytokines profile might regulate the balance of CTLs and Th17 cells. However, this hypothesis requires further investigation.

Another interesting characteristic of Th17 cells is their ability to produce IL-22. Because IL-22 has a protective role for hepatocytes in a *concanavalin A-induced mouse hepatitis* model [12], it would be interesting to investigate how IL-22 and IL-17 produced by Th17 cells relate to the pathogenesis of AIH.

1.4.4.3 Natural Killer T Cells

Invariant natural killer T (iNKT) cells are unique because they express the T-cell receptor (TCR) as well as NK-cell receptor and are constitutively distributed among several organs, including the liver where iNKT cells represent >50 % of the intrahepatic lymphocyte population. Because iNKT cells produce Th1 and Th2 cytokines during certain immune responses, they may act as effector or regulatory cells in the pathogenesis of autoimmune diseases.

iNKT cells work as effector cells to damage hepatocytes in a concanavalin A-induced mouse hepatitis model. However, it remains unknown whether iNKT cells also work as effecter cells in human AIH.

1.5 Regulatory T Cells in the Pathogenesis of AIH

In general, autoreactive T cells are eliminated in the thymus. However, some autoreactive T cells escape elimination and exist in the peripheral blood of healthy people. Such autoreactive T cells are suppressed by regulatory T cells such as Foxp3-positive regulatory T cells (Tregs) or Tr cells, which produce IL-10, in peripheral blood (Fig. 1.6). This mechanism is called "peripheral tolerance," which protects against autoimmune diseases under normal physiological conditions. However, if the number or function of regulatory cells altered, negative regulation of autoreactive T cells may be lost, thereby leading to development of autoimmune diseases. In fact, a decrease in the number of Tregs and dysfunction of Tregs has been reported in several autoimmune diseases.

1.5.1 Frequency and Function of Tregs

In AIH, a decrease in the number of peripheral blood Tregs at diagnosis compared with healthy controls has been reported [13]. Interestingly, recovery of the number of Tregs during remission has also been reported [14]. Furthermore, a loss of suppressive function of CD8-positive cells at diagnosis as well as recovery during remission has been reported [15]. These results indicate that a decrease in the number of Tregs and dysfunction of Tregs at diagnosis correlates with the onset of AIH.

These data remain controversial because another study reported that Tregs in AIH were fully functional and not reduced in number [16]. Further study is needed to resolve the role of Tregs in AIH.

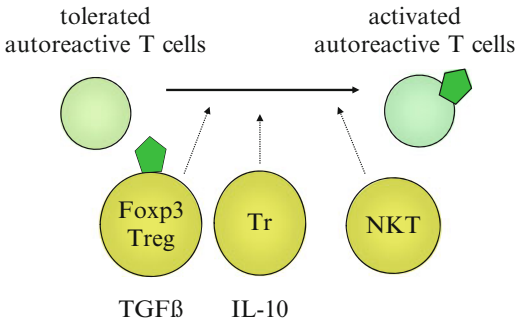

Fig. 1.6 Regulatory cell subsets that maintain peripheral tolerance

1.5.2 Natural Tregs and Inducible Tregs

There are two subsets of Tregs, *natural Tregs* (nTregs; differentiated in the thymus) and *inducible Tregs* (iTregs; induced from naïve CD4-positive, Foxp3-negative T cells), in peripheral blood or an organ in the context of inflammation. TGF-β is considered an important factor for inducing iTregs.

Because iTregs are induced in the context of inflammation, it is possible that they suppress inflammation. In an experimental mouse AIH model, which was established by injecting a fusion cell of DCs and well-differentiated hepatoma cells, an increased expression of Foxp3-positive iTregs was observed in the inflamed liver [17]. Furthermore, inflammation decreased after the expression of iTregs, suggesting that iTregs induced in the inflammatory phase work as negative regulators of inflammation [17].

However, a recent study revealed that both the expression of Foxp3 and the induction of a specific *DNA methylation pattern* are required for the regulatory function of Tregs [18]. Although nTregs have both properties and demonstrate regulatory functions, some iTregs do not have the Treg-specific DNA methylation pattern although they express Foxp3. Such iTregs do not demonstrate regulatory function. These findings indicate that iTregs, which become Foxp3 positive in the context of inflammation, possibly cannot function as a true "Treg" because they do not have the Treg-specific DNA methylation pattern. Thus, great care should be taken to carefully evaluate the significance of iTregs in the inflamed liver.

1.5.3 Balance of Tregs and Th17 Cells

Recent reports have revealed that a shift in the quantitative balance of Tregs and Th17 cells, resulting in a predominance of Th17 cells, is observed in PBC [19]. Interestingly, TGF-β is required for the differentiation of both Tregs and Th17 cells. However, IL-6 is required for the differentiation of Th17 cells but not for that of Tregs (Fig. 1.7). These observations indicate that the balance of Tregs and Th17 cells may have a role in the pathogenesis of AIH primary by regulating the dominance of effector cells and regulatory cells and that the cytokine profile may prescribe this balance.

1.6 Intrahepatic Immunological Status

Recent reports suggest that immune cells, hepatocytes, and non-parenchymal cells (*Kupffer cell*, *hepatic stellate cell*, and *sinusoidal endothelial cell*) in the inflamed liver participate in the pathogenesis of AIH by expressing several important functional molecules and interacting with lymphocyte (Fig. 1.8).

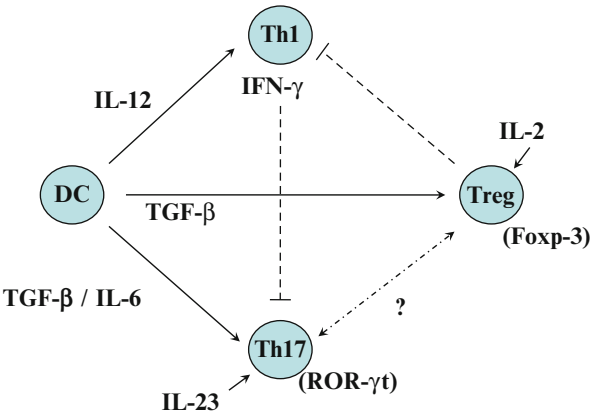

Fig. 1.7 Cross talk between Th1 cells, Th 17 cells, and Tregs

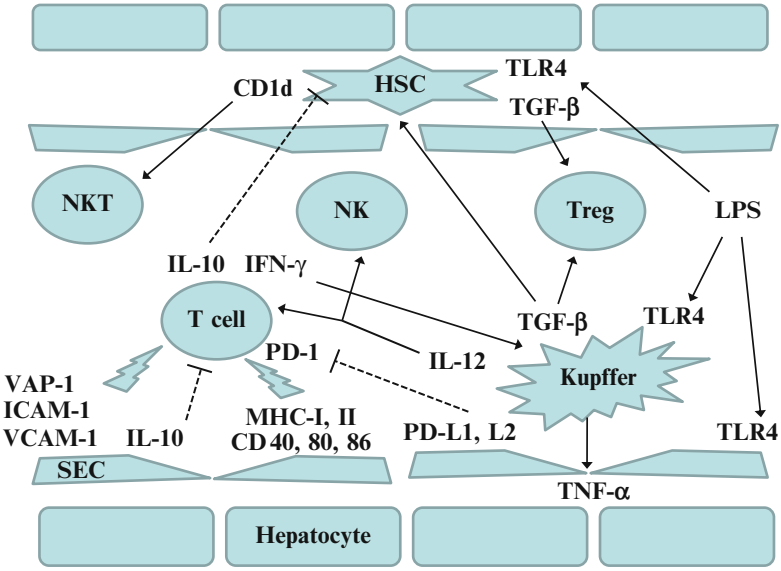

Fig. 1.8 Interaction between immune cells and non-parenchymal cells, which promote intrahepatic immunological status that participates in the pathogenesis of AIH

1.6.1 Recruitment of Lymphocytes

When sinusoidal endothelial cells express adhesion molecules such as *ICAM-1* and *VCAM-1*, which are induced by inflammatory cytokines present in the inflamed liver, the ligands of these molecules expressed by lymphocytes could attach to sinusoidal endothelial cells and migrate into the liver from peripheral blood. Because the expression of adhesion molecules is induced by inflammatory

cytokines such as IL-12, such phenomenon may occur in the inflamed liver. In addition, inflammatory cytokines may also induce the production of *chemokines* by intrahepatic immune cells or non-parenchymal cells, which participate in the recruitment of lymphocytes. Because inflammatory cytokines also induce the expression of chemokine receptors, lymphocytes could migrate easily into the site of inflammation. Therefore, to understand the mechanisms of intrahepatic recruitment of lymphocytes, it is important to analyze which *non-parenchymal cells* express which adhesion molecules as well as which cells produce which chemokines in the inflamed liver. Moreover, because a particular subset of lymphocytes responds to specific adhesion molecules and chemokines, it is also important to know the mechanisms of lymphocyte subset-specific intrahepatic recruitment.

1.6.2 Interactions of Non-parenchymal Cells, Hepatocytes, and Immune Cells

In addition to recruitment of lymphocytes, non-parenchymal cells and hepatocytes may influence the function of immune cells. For example, when non-parenchymal cells and hepatocytes express the ligand of co-stimulatory molecules, they can regulate the function of immune cells by interacting with co-stimulatory molecules expressed on the immune cells. If non-parenchymal cells and hepatocytes express B7-2 or PD-L1, which are the ligands of inhibitory co-stimulatory molecules CTLA-4 and *PD-1*, they can inhibit activated intrahepatic lymphocytes and regulate inflammation. In AIH, an increased expression of PD-1 on intrahepatic T cells and PD-L1 on Kupffer cells and sinusoidal endothelial cells has been reported [20]. The same phenomenon was also observed in type C chronic viral hepatitis. Because the expression of inhibitory co-stimulatory molecules and their ligands is induced by inflammatory cytokines, it is possible that an increased expression of inhibitory co-stimulatory molecules and their ligands, in the context of inflammation, may protect against excessive inflammation. However, it is incorrect to make such an assumption based on the analysis of only one pathway of co-stimulatory molecules because many types of co-stimulatory molecules and their ligands have been recently identified. In addition, the influence of interactions between non-parenchymal cells, hepatocytes, and immune cells through co-stimulatory molecules might be very complicated because there are two types of co-stimulatory molecules. One type can activate immune cells, whereas the other has an inhibitory function. Thus, it may be very important to comprehensively analyze these effects.

1.6.3 Interaction of Immune Cells

Several types of immune cells such as T cells, NK cells, NKT cells, Tregs, and Kupffer cells exist in the liver. Indeed, the liver has a high frequency of cells involved in innate immunity, such as NKT cells and NK cells, compared with other organs. Therefore, it is possible that there is interaction between immune cells in the inflamed liver, for example, Kupffer cells may activate T cells, NK cells, or Tregs by producing *IL-12* or TGF-β.

Many types of cells and factors participate in promoting intrahepatic immunological status, which directly contributes to the pathogenesis of AIH. Methods must be developed to evaluate the interaction of several cells and molecules.

1.7 Conclusion

As this review clearly points out, there are a lot of unanswered questions regarding the pathogenesis of AIH. At this juncture, perhaps the most important step is to identify the autoantigen. In addition, development of methods is needed for further assessing the role of multiple cell types and their interactions in the pathogenesis of AIH.

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Chapter 2
Models of Autoimmune Hepatitis

Norihiko Watanabe and Aki Ikeda

Abstract Several murine models of autoimmune hepatitis (AIH) have been described in the course of investigating the immune mechanisms involved in the development of AIH. However, those models have lacked the characteristics of human AIH, such as hypergammaglobulinemia and production of circulating autoantibodies. In contrast, we have developed mouse models of spontaneous AIH with hypergammaglobulinemia and ANA production. Immune dysregulation by a concurrent loss of naturally arising regulatory T cells and PD-1-mediated signaling induces different phenotypes of AIH in mice with different genetic backgrounds. Using these models, we have shown that the spleen is the induction site for AIH, that follicular helper T cells constitute the T-cell subset responsible for induction, and that the CCR6–CCL20 axis is crucial for the migration of dysregulated T cells from the spleen into the liver. As fatal AIH progresses, the CXCR3–CXCL9 axis is crucial for the migration of T helper 1 cells and effector $CD8^+$ T cells into the liver, causing fatal damage. Dendritic-cell-derived IL-18 is critical for differentiation of CXCR3-expressing Th1 cells and $CD8^+$ effector T cells in the spleen. In addition, we have found some clues that should help in overcoming the therapeutic insufficiency of corticosteroids for AIH patients.

Keywords Autoimmune liver disease • Chemokines • Cytokines • Pathogenesis • T-cell response

N. Watanabe (✉) • A. Ikeda
Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho Sakyo-ku, Kyoto 606-8501, Japan
e-mail: norihiko@kuhp.kyoto-u.ac.jp

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2.1 Introduction

Autoimmune hepatitis (AIH) is an immune-mediated disorder in the liver that is characterized by the histological findings of mononuclear cell infiltration invading the parenchyma, ranging from piecemeal necrosis to submassive lobular necrosis without bile duct destruction in the portal area [1, 2]. In addition, the serologic hallmarks of AIH are elevated gamma globulins and the production of a variety of characteristic circulating autoantibodies (autoAbs), including antinuclear antibodies (ANAs) [1, 2]. There is little evidence that autoAbs play a part in the pathogenesis of AIH; liver-infiltrating T cells are considered to be the primary disease mediators of inflammatory liver damage in AIH [3, 4]. However, it had been unclear how an aberrant regulation of autoreactive T cells against liver antigens is initiated in vivo and how the dysregulated autoreactive T cells trigger the development of AIH. In addition, it had been uncertain how characteristic B-cell activation, including B-cell mediated autoimmunity, is associated with its development.

Several murine models of AIH have been described in the course of investigating the immune mechanisms in the development of AIH [4]. In those models, concanavalin A (Con A)-induced acute hepatic injury, associated with activation of natural killer T cells and T cells, has been extensively examined as an experimental model of human AIH [4–6]. However, Con A-induced acute hepatic injury does not produce circulating autoAbs; a single intravenous injection of Con A into BALB/c mice induces rapid injury of hepatocytes, with a striking increase in plasma transaminase levels within 24 h [5, 7]. Thus, this model is not likely to provide clues for understanding the molecular mechanisms that trigger AIH development.

Recently, we have developed mouse models of spontaneous AIH [8, 9]. Using these models, we have identified induction sites, responsible T-cell subsets, and key molecules for induction of AIH and described the mechanisms involved in the progression to fatal AIH [10–13]. In addition, we have found some clues to revealing varied clinical manifestations of AIH and overcoming the therapeutic insufficiency of corticosteroids for AIH patients [9].

2.2 A New Mouse Model of Spontaneous AIH

Naturally arising Foxp3$^+$ regulatory T (Treg) cells are critical in maintaining immunologic self-tolerance and in regulating a variety of pathological and physiological immune responses [14]. In patients with AIH, Treg cells are reduced numerically and functionally, and Foxp3 expression of Treg cells is lower than in normal subjects [15, 16]. However, loss-of-function mutations in the gene encoding Foxp3 in mice and humans and severe Treg-cell depletion by neonatal thymectomy (NTx) of normal mice result in the development of organ-specific autoimmune